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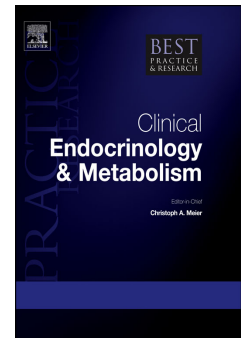
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Single embryo transfer: why and how to identify the embryo with the best developmental potential

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Abstract

Multiple pregnancies with higher risk of preterm birth and the associated higher morbidity have been a major obstacle from the early days of in vitro fertilization. A good strategy to avoid multiple pregnancies is elective single embryo transfer and cryopreservation of spare embryos. Important factors in adopting this strategy are good counselling of the patients and the selection of embryos with high implantation potential. Technical advances in embryo selection have been described during recent years, time lapse monitoring and genetic assessment of the embryos being the most important achievements. With these studies we have gained new information on early embryos. However, at present, there is insufficient evidence to recommend the routine use of these new techniques. The ultimate goal of infertility treatment is a healthy baby.

Key words

Assisted reproduction technology (ART), in vitro fertilization (IVF), cryopreservation, elective single embryo transfer (eSET), time lapse monitoring, preimplantation genetic testing for aneuploidy (PGT-A)

A. Introduction

More than seven million children have been born worldwide as the result of assisted reproduction technology (ART). Multiple pregnancies with higher risk of preterm birth and the associated higher morbidity have been a major obstacle from the early days of IVF (1). The high multiple pregnancy rate of ART is a significant public health issue and, as a result, single embryo transfer (SET) should be encouraged. However, SET also demands more consistent methods for identifying the best embryo for transfer, as well as improved methods for predicting live-birth.

However, SET is not the only solution to the adverse perinatal outcome in IVF children, as growing evidence has shown that ART singletons also have a slightly higher risk of preterm birth, low-birth weight, being small for gestational age, and an increased rate of congenital malformations (2, 3).

Editorial comment:

Recently, higher neonatal birth weight was reported after transfer of frozen thawed embryos. Ref.: Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod.* 2014 Mar;29(3):618-27.

ESET is leading to more transfers of previously frozen embryos. So in contrast to the current situation, in which the birth weight of newborn children is somewhat lower, if they were conceived in a fresh cycle, we are moving towards the opposite situation. Shouldn't we add this to the review?

The causes of the poorer outcome in ART singletons are probably multifactorial. Subfertility is a major risk factor for adverse perinatal outcome in ART singletons, however, even in the same mother an ART singleton has a poorer outcome than the non-ART sibling; hence, factors related to the hormone stimulation and/or IVF methods per se also may play a part (1). The major concern is whether the IVF techniques themselves could have negative impacts on the ART offspring.

To encourage elective SET, several methods have been studied to identify and select the best embryo for transfer, such as blastocyst culture, time-lapse imaging and preimplantation

genetic testing. A trend of increasing blastocyst transfers combined with improvements in embryo selection techniques results in increases in elective SET. It also allows clinics to more confidently offer SET (4).

Despite continuing technical advances, however, it is likely that small but potentially significant live-birth rate differences will persist between single and double embryo transfer if we continue to emphasize pregnancy rates per transfer instead of cumulative pregnancy rates (derived from the fresh and frozen cycles) per oocyte pick-up. Predictive models suggest that cumulative live-birth rates with the use of sequential SET are equal or superior to DET (5). Further studies confirming this prediction will help to convince physicians, patients, and health care providers of the benefits and feasibility of SET, even if this strategy requires additional transfers and a slightly longer time to pregnancy.

A. How elective single embryo transfer has evolved

The implementation of eSET in different countries has occurred in different ways. In Finland the initiative for reducing the number of transferred embryos came from the IVF clinics taking the responsibility for the safety of ART, initially when treating high-risk patients. The results of the preliminary experience convinced to continue and eSET strategy was accepted gradually nationwide (6). In Sweden the implementation of eSET in a larger scale occurred following the new rules from the National Board of Health and Welfare, which instructed that in ART only one embryo should be transferred, except in cases where the risk for a twin pregnancy is estimated to be low. The implementation of SET in Sweden was easier than one had imagined (7). There is another kind of example from Belgium where eSET was introduced in few large infertility centres since 1998 (8). From July 2003 onwards a reimbursement system for six IVF/ICSI cycles linked to the transfer policy has been set up, based on the clinical experience of Belgian groups (9). The arguments behind this agreement were diminished neonatal costs as result of reducing the twin pregnancy rate to half and the high order multiples to almost zero.

A consortium organized by ESHRE, named EIM (European IVF Monitoring), includes representatives from the European countries participating in data collection. In the analysis of the first 15 years of EIM activity a clear trend in transferring fewer embryos has been continuously observed over the years, despite some differences among countries (10).

Already in 2000, double embryo transfers became the most frequently used in Europe. At the same time, the percentage of single embryo transfers (SETs) started to increase, exceeding the number of three embryo transfers in 2005. The EIM reports are unable to discriminate between elective and compulsory SET, however, the increase seen in the more recent years is due to an overall increase of the eSET policy. When reducing the number of embryos transferred, the number of cryopreservation cycles increases, as also found in this report. As expected, the number of multiple deliveries (twins, triplets and more) decreased from 29.5% to 19.2% during the 15 years. The best indicator of success, the cumulative delivery rate, unfortunately cannot be calculated exactly based on the annual EIM reports (10).

Also in US a marked and linear reduction in multiple birth rates has been seen, and importantly, little to no effect on clinic-level live birth rates (4). From this analysis it would appear that quite high rates of eSET are required if one is to effectively address the complication of multiple births after IVF. The higher acceptance of eSET is lies on improvements in embryo cryopreservation and embryo genetic analysis and selection. A study from US reports a 10%–15% reduction in live birth rate and at least a 47% decrease in MBR with SET compared with DET in the setting of favourable patient prognostic factors, including younger age, transfer of a blastocyst, and additional embryos cryopreserved (11).

A. Risks of multiple pregnancies

Multiple pregnancies are associated with considerable risks for the mother and offspring as well as excess obstetric and neonatal costs (12). Of these risks, prematurity is the most important; indeed, multiple pregnancy is associated with a six-fold increase in the risk of preterm birth. In general, an increased risk of neurological sequelae occurs in multiple pregnancies. The risk for cerebral palsy even is five-to ten-fold. In addition, prenatal and neonatal complications can result in health problems later in the children's lives, including mental and physical disabilities (13). The cost of care for children born prematurely as a result of multiple births is also considerable (14).

Many risks to the health of the mother are well documented. Maternal complications include increased risk of pregnancy-induced hypertension, pre-eclampsia, gestational diabetes, obstetric haemorrhage, and operative delivery. Maternal morbidity is related to the number of foetuses. Perinatal complications increase morbidity of neonates from twin pregnancies.

Mortality rates are also increased: stillbirths, early neonatal, late neonatal and infant mortality are higher in multiple pregnancies, increasing with the number of fetuses. Preterm delivery and low birth weight are the main factors accounting for the excess in neonatal morbidity. Many multiple gestation neonates require treatment and extended care in neonatal intensive care units, and about one-half of twins and 80% of triplets are admitted to neonatal intensive care units.

The psychosocial aspects are important, too. The transition to parenthood is a period of change and stress, and even more for the parents of twins. They have to cope with two infants whose needs may be demanding as a result of prematurity, perinatal complications or disability. This may cause feelings of insufficiency and uncertainty, and can lead to increased parental stress, depression and anxiety. In a Finnish study, twin parenthood, but not ART, had a negative effect on the mental health of both mothers and fathers during the transition to parenthood (15).

A. Prevention of multiple pregnancies in assisted **reproduction**

The practice of eSET programme, which consists of transferring one single fresh embryo, followed by one or more frozen–thawed embryo transfer cycle(s) as needed, has reduced multiple pregnancy rates while maintaining acceptable cumulative live birth rates (12). Considerations of safety encouraged many clinics to adopt a policy of eSET. This policy was based on continued refinement of all stages of treatment, including patient counselling, ovarian stimulation, laboratory conditions and embryo selection. The evaluation of cumulative delivery rates becomes more relevant, taking into account both efficacy and safety. In a recent Swedish population-based registry study cumulative delivery rate per aspiration increased up to 20 oocytes retrieved and then evened out while the incidence of severe OHSS increased more rapidly from around 18 oocytes and thromboembolic events, although rare, occurred in particular if 15 or more oocytes are retrieved. The principal conclusions by the authors of this study were that live birth delivery rate after fresh cycles increased when up to 11 oocytes were retrieved and then levelled off while the cumulative delivery rate per aspiration rose up to at least 20 oocytes retrieved (16).

B. Oocyte quality

Success of ART has for long been focused on technical aspects of treatment: the number of follicles and oocytes, the fertilisation and cleavage rate. In treatment cycles in which ovarian stimulation in conjunction with IVF, ICSI, or both, results in a large number of fertilised oocytes, cryopreservation offers the opportunity to select the best embryo for fresh transfer and to freeze the others for later use. This optimises the clinical use of available good quality embryos. In general, a good stimulation is one that produces a homogeneous cohort of mature oocytes, with the least inconvenience and risk to the patient, and results in the birth of a healthy singleton (17).

Non-invasive selection of developmentally competent human oocytes may increase the overall efficiency of human assisted reproduction. In a review Swain and Pool (18) summarize the short list of features of an oocyte that is regarded healthy on the basis of morphological investigations during the routine IVF programme: single polar body, normal-looking cytoplasm, appropriate zona pellucida thickness, proper perivitelline space. According to the authors, the common experience is still that these features often fail to predict the future fertilizing ability and developmental competence. The following structures can further be investigated: meiotic spindle, vacuoles or refractile bodies, polar body shape, oocyte shape, dark cytoplasm or diffuse granulation, central cytoplasmic granulation, cumulus-oocyte complex and cytoplasm viscosity and membrane resistance characteristics (19). However, no clear tendency in recent publications to a general increase in predictive value of these morphological features was found.

B. Embryology

Morphological assessment of preimplantation stage embryos is still one key element of the embryology laboratory work (20). Traditionally, identifying embryos with highest potential for implantation has focused on prediction models based on morphological characteristics where routine inverted microscopic investigations are performed at predetermined checkpoints. At the zygote state, certain patterns of pronuclei (number and the distribution of nucleoli) have been found to correlate with treatment outcome in IVF and ICSI cycles, offering a possible prognostic tool prior to cleavage. However, it is not known whether indicators referring to zygote morphology are useful, especially as differences in pronuclear pattern could be related to the insemination method and timing of the observation (17).

Proposed indicators for cleavage-stage embryos are early cleavage rate, cleavage rate, embryo development rates, embryo fragmentation rate, and rate of good quality embryos (embryo score or grade). Studies have shown that early cleavage, together with other factors, can be used as an embryo selection method (17). A classical study (21) characterized a top quality embryo as follows: absence of multinucleated blastomeres, four or five blastomeres on day 2, seven or more cells on day 3, and $\leq 20\%$ anucleated fragments. A large prospective SET study performed with the aim of constructing an evidence-based embryo score for the ranking and selection of early cleavage stage embryos shows that blastomere number, proportion of mononucleated blastomeres, and degree of fragmentation size have independent prognostic power to predict live birth (22).

Delaying transfer and prolonging embryo culture to the blastocyst stage is argued to improve uterine and embryonic synchronicity. Assessment of blastocyst morphology is straightforward in the case of good quality blastocysts, but can be challenging for embryos showing an attempted cavitation (17). Blastocyst quality should be based on blastocoele expansion, appearance of trophectoderm, and appearance of inner cell mass. In a Swedish study, all three parameters had a significant effect on live birth. However, once adjusted for known significant confounders, it was shown that appearance of trophectoderm was the only statistically significant independent predictor of live birth outcome (23). It may be that, even though inner cell mass is important, a strong trophectoderm layer is essential at this stage of embryo development, allowing successful hatching and implantation.

In another study Van den Abbeel et al. (24) assessed the ability of three individual blastocyst morphology parameters - expansion and hatching stage, inner cell mass grade and trophectoderm grade - to predict outcome of a cycle with single-blastocyst transfer. In the simple logistic regression analysis, all three blastocyst morphology parameters were statistically significantly associated with clinical and ongoing pregnancy rates and live birth rates, while only the inner cell mass grade was significantly associated with early pregnancy loss rate. Blastocyst expansion and hatching stage was the only significant predictor of live birth in the multiple logistic regression. In conclusion, although all three blastocyst morphology parameters were related to treatment outcome of fresh single-blastocyst cycles, selection of high-quality blastocysts for transfer should consider first the expansion and hatching stage (24).

B. Cryopreservation

The number of cryopreservation cycles was fairly constant in Europe during the 1990s, but has increased over the past 20 years. Many variables may influence the outcome of embryo cryopreservation and frozen embryo transfer. Several reports indicate that transfer of partially damaged thawed embryos results in lower pregnancy rates compared with transfer of fully intact embryos. Successful eSET program needs high quality cryopreservation techniques. Over the past decade vitrification has proved to be a successful method of blastocyst cryopreservation in human IVF, achieving high pregnancy and live birth rates. When transferring cryopreserved blastocysts, pre-freeze blastocoele expansion and trophectoderm grade and post-thaw degree of re-expansion seem to be the most significant predictors of live birth in frozen-thawed blastocyst transfer cycles (25).

A. New possibilities for embryo selection

B. Metabolomics

There is a need for secondary measures in addition to morphology that will provide precise physiological information about an individual embryo to facilitate selection for SET.

Metabolomics has been proposed as a non-invasive method to assess oocyte quality, embryo viability, and endometrial receptivity (26). Metabolomics is defined as the non-targeted identification and quantification of all low molecular weight end-products of metabolism (metabolites). It reflects events well downstream of gene expression and gives valuable information about the metabolism within cells, that other 'omics' technologies cannot (27).

The premise of any technology examining metabolic, metabolomic or proteomic differences between embryos is that this information provides a greater ability to establish the viable embryo within a cohort of good quality embryos. Near infrared (NIR) spectroscopy is a technology proposed to facilitate non-invasive screening for the most optimal human embryo for uterine transfer. It has been proposed that the NIR spectral profile of an embryo's spent culture medium can be used to predict the implantation potential and can serve as a method to compare and select between sibling embryos. As the initial proof of principle studies were all retrospective, NIR spectroscopy on spent embryo culture medium was investigated in an on-site, prospective setting could improve the ongoing SET pregnancy rate after Day 2 and 5

transfers. The study was terminated early as the analysis of the Data Safety Monitoring Board showed a very low conditional power of superiority for the primary outcome (28). Thus their conclusion was that the addition of NIR spectroscopy to embryo morphology does not improve the chance of a viable pregnancy when performing SET, and in its current form cannot be used as an objective marker of embryo viability. The study by Kirkegaard et al (29) also questions the usefulness of the entire metabolome for embryo selection, which should direct the search for viability markers in the culture media towards individual components.

The aim of a recent systematic review was to examine the potential application of metabolomics to female reproduction, specifically to the metabolomics of follicular fluid, embryo culture medium and endometrial fluid (30). According to this review there is some weak evidence that metabolomics technologies studying embryo culture media might be able to predict the viability of individual embryos better than standard embryo morphology. However the conclusion was that there is currently no evidence that metabolomics profiling can improve fertility outcomes (30).

All randomised controlled trials (RCTs) on metabolomic assessment of oocyte quality, embryo viability, and endometrial receptivity in women undergoing ART were analysed also in Cochrane review. They came to the same conclusion: at the moment there is no evidence to support or refute the use of this technique for subfertile women undergoing ART (26).

B. The potential of time lapse embryo culture to improve outcomes

Embryo incubation and assessment is a vital step in ART. In 2009, the first automatic time-lapse devices became commercially available for ART clinics, moving from very primitive cinematography to a massive analysis of growing embryos in a few hours (31). With the introduction of time-lapse imaging, where an image of each embryo is taken every 10 to 20 minutes, more embryo parameters can be viewed while leaving the embryos in an undisturbed environment. There are also more commercial interests becoming involved in the production of equipment (32).

Traditionally, embryo assessment has been achieved by removing embryos from a conventional incubator for assessment of under a light microscope daily. Time-lapse systems taking digital images of embryos at frequent time intervals allow embryologists to assess the

quality of the embryos without physically removing them from the incubator. Stable culture environment limits the exposure of embryos to changes in gas composition, temperature and movement. More frequent observations will provide substantially more information on the relationship between development, timing, and embryo viability (33). This could afford potential advantage of improving single embryo selection for ART treatment by utilising additional information gained through monitoring embryo development (34). However, this review concluded, that there is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between time-lapse system and conventional incubation.

As the availability of time-lapse technologies increased, attention was first focused on assessing their clinical safety. Once the safety had been established and the available technologies were validated for clinical use, research then turned to determining how the time-lapse imaging systems could be used to increase pregnancy rates through in-depth embryo analysis and an undisturbed culture system (35).

Standardized timing of observations is critical. As morphology and developmental competence is not firmly correlated, morphological assessment has limited predictive value in the identification of the most viable embryos. To determine whether incubation in the integrated EmbryoScope time-lapse monitoring system (TMS) and selection supported by the use of a multivariable morphokinetic model improve reproductive outcomes in comparison with incubation in a standard incubator and selection based exclusively on morphology was studied in a prospective, randomized, double-blinded, controlled study (36). They concluded that the strategy of culturing and selecting embryos in the integrated EmbryoScope time-lapse monitoring system improves reproductive outcomes. The ongoing pregnancy rate was statistically significantly increased 51.4% for the TMS group compared with 41.7% for the standard group (36). However, the project was unable to demonstrate whether the improvement were from the unchanging culture conditions provided by the EmbryoScope or the morphokinetic embryo selection algorithm applied (31).

The pilot randomized controlled trial (RCT) published by Kaser et al. (37) concluded that adjunctive use of another time-lapse system, the Eeva test, did not improve clinical pregnancy rate per transfer. However, this study compared day 3 single embryo transfer with Eeva test to day 5 single embryo transfer with conventional assessment alone, but did not compare day 3

transfer with or without Eeva. In contrast, the study by Adamson et al. (38) is one of the few studies to directly compare day 3 embryo transfers using a TLM system against day 3 transfers with conventional morphology analysis alone, and demonstrated significantly higher implantation and clinical pregnancy rates in the test group compared with controls.

In contrast, in a study by Goodman et al (35) the addition of time-lapse morphokinetic data did not significantly improve clinical reproductive outcomes. Clinical pregnancy and implantation rates were similar overall and with blastocyst transfers. Clinical pregnancy rate with day 5 transfer was threefold higher than day 3 transfer, but the use of time-lapse system was not a significant predictor of implantation. In addition, the authors confirmed that the time of the start of blastulation, the absence of multinucleation and the use of a score based on morphology were significant predictors of implantation (35).

Time-lapse monitoring has emerged as a novel technology to perform semi-quantitative evaluation of embryo morphology and developmental kinetics in assisted reproduction. While this method has already been introduced into clinical practice in many laboratories, it is unclear whether it adds value to conventional morphology (39). To study the efficacy of six embryo-selection algorithms when applied to a large, exclusive set of known implantation embryos a retrospective, observational analysis was carried out (40). Their data suggested that currently available embryo-selection algorithms may not be clinically applicable and lose their diagnostic value when externally applied.

The remaining questions are: is blastocyst prediction clinically useful; is there any morphokinetic algorithm available for all time-lapse devices; can it be sold as a diagnostic test; regarding embryo euploidy, can it be forecast by a non-invasive morphokinetic test; and is time-lapse useful for selecting euploid embryos? (31). As more data become available, it is of the utmost importance that groups using time-lapse methods share a common nomenclature for measured time points. It has been proposed that until such evidence accumulates, selection of embryos by time-lapse should remain an experimental strategy subject to institutional review and approval (39). One thing seems certain: as technology advances, the debate about time-lapse will not remain static. With time and experience, we may well come to quite different conclusions (41).

B. The potential of genetic assessment of the embryo to improve the outcomes further.

During recent years one important focus has been on analysis of all 24 chromosome copy number for evaluation and transfer of only euploid embryos, now known as preimplantation genetic testing for aneuploidy (PGT-A) (also known as preimplantation genetic screening; PGS) (42). Initially, PGT-A was performed principally on day 3 embryos, whereby a single blastomere was biopsied and analysed with fluorescence in situ hybridization (FISH). This was followed two days later with a blastocyst transfer. It took almost a decade after its clinical introduction before the first properly designed RCTs on this technique were published. Astonishingly, these did not indicate a benefit of PGT-A, but a significantly decreased chance on ongoing pregnancy in comparison with IVF without PGT-A (42).

The Blastocyst Euploid Selective Transfer (BEST) trial enrolled IVF couples with a female partner up to age 42 years. They were randomized to receive transfer of a single euploid embryo (eSET) or to the standard of care with transfer of two embryos that were not biopsied (untested double embryo transfer). Among the 175 randomized patients, the delivery rates were similar through the fresh cycle and up to one frozen transfer (69% after euploid eSET vs 72% after untested DET), with a dramatic difference in multiple births (1.6% vs 47%) (43).

More recently, several randomized controlled studies produced evidence that day 3 biopsy with PGT-A is detrimental to outcome, partially due to its invasive nature and to embryo mosaicism (44). The mosaic nature of human preimplantation embryos at this developmental stage and the technique used, FISH analysis of a limited number of chromosomes, are considered the main reasons for the inefficacy of PGT-A (42). Newer techniques are substituting FISH: comparative genomic hybridization (CGH), array CGH (aCGH), digital polymerase chain reaction (dPCR), single-nucleotide polymorphism (SNP) array, real-time quantitative PCR (qPCR), and next-generation sequencing (NGS) (45). Although NGS has the advantage of high accuracy and decreased cost compared with aCGH, more research is needed to elucidate the mosaicism phenomenon further (45). Mosaicism refers to two or more cell populations with different chromosomal complements being present within the same embryo. Embryonic mosaicism is believed to be a confounder when trying to interpret PGT-A results.

A clinical scenario in which PGT-A may be of significant benefit is to increase utilization of eSET (45). A 2015 study compared IVF success before and after a change in clinic protocol

designed to decrease the number of embryos transferred in patients older than 35 years (Ubaldi). Elective SET was offered in patients with fewer than two implantation failures if favourable embryo morphology and/or PGT-A screening occurred. There were no significant differences in clinical pregnancy rates per transfer pre- and post-change in protocol, but there was a significant increase in live-birth rates per embryo transfer cycle for the eSET-PGT-A recipients. However, only 43.6% of PGT-A cycles had at least one euploid embryo to transfer. When comparing live-birth rates per cycle, there was no significant difference between groups (20.9% without PGT-A vs. 24.4% with PGT-A) (46)

The goal of the study by Kang et al. (44) was to compare IVF outcomes between women undergoing frozen transfers of euploid blastocysts after PGT-A with patients undergoing fresh blastocyst transfers without PGT-A to determine whether routine application of this technology proves beneficial. They found that among women 37 years old or younger, IVF-PGT-A does not improve live-birth rates, nor does it reduce miscarriage rates. Among women older than 37 years, IVF-PGT-A does improve clinical pregnancy and live-birth rates when the data are analysed per ET. However, when the data are analysed per retrieval, this advantage does not persist (44). And they conclude: the universal application of PGT-A adds extra cost and invasiveness with limited benefit, especially in younger women.

ASRM states recently (45): The value of preimplantation genetic testing for aneuploidy (PGT-A) as a screening test for IVF patients has yet to be determined. Several studies demonstrate higher birth rates after aneuploidy testing and eSET, suggesting the potential for this testing to decrease the risk of multiple gestations, though these studies have important limitations. At present, however, there is insufficient evidence to recommend the routine use of blastocyst biopsy with aneuploidy testing in all infertile patients.

A. eSET and pregnancy outcome - The Finnish experience

In Finland the implementation of eSET started in 1997. In the beginning this was recommended to women in whom a twin pregnancy could be predicted to carry high obstetric risks. The results of eSET in these special groups of patients were encouraging (47). The results of these initial data revealed a different prognosis between eSET and cases of SET when only one embryo was available. In a prospective randomized study carried out in three large clinics in Finland, the pregnancy rate per transfer after eSET was 32% and cumulative

pregnancy rate per patient after the transfer of fresh and frozen embryos was 47.3% (48). In Helsinki University Hospital we analysed in a follow-up study all embryo transfers carried out at our clinic between 1998 and 1999. The contribution of embryo cryopreservation in eSET cycles resulted in the cumulative delivery rate of 52.8% per oocyte retrieval after fresh and frozen transfers (49). Indeed, we could report halving of twinning rates while maintaining very acceptable pregnancy rates (50). This was possible with by improving embryo selection methods and acknowledging the benefit of embryo cryopreservation on the cumulative pregnancy rates. It is obvious that a significant effect of eSET is an increase in the number and quality of embryos available for cryopreservation. We also showed that SET policy can be adopted into cryopreservation programme (51).

In Finland the initiative for reducing the number of transferred embryos came first from a few IVF clinics. Our experience confirms that the practice of reducing the number of transferred embryos can be implemented not only among single clinics, but also across the whole country. The decrease in multiple deliveries results in an improvement of the perinatal health of the IVF children, thus it is also important to consider the overall beneficial health-economic impact. Nowadays, in Finland, SET is practiced in 80% of the cycles and even 100% in some clinics.

According to the Finnish IVF registry the proportion of single-embryo transfers has increased steadily every year since 1997, both in fresh and frozen embryo transfers (Fig 1). The effect of this is reflected in the significant decrease in the proportion of twin deliveries after ART in our country (Fig 2) as well as in the proportion of multiple deliveries in the whole country (Fig 3). This means that most ART children are born full-term (Fig 4).

A. Conclusion

The clinical risks to mothers and babies associated with ART multiple birth pregnancies are well described and widely recognized. A meta-analysis of individual patient data from randomized trials shows that elective SET results in a higher chance of delivering a term singleton live birth compared with double embryo transfer. Although this strategy yields a lower pregnancy rate than a double embryo transfer in a fresh IVF cycle, this difference is almost completely overcome by the addition of a subsequent frozen SET cycle (52). The

strategy to a large extent overcomes the problems associated with multiple pregnancies and is now used in many countries.

Nordic population-based matched cohort study based on data from more than 92 000 ART children shows that the perinatal outcomes after ART have improved over the last 20 years, mainly due to the reduction of multiple births. Also for singletons conceived after ART, a remarkable decline in the risk of being born preterm and very preterm was observed. Data from these four countries confirm an overall improvement over time in the perinatal outcomes of ART children. Especially this study show the beneficial effect of single embryo transfer, not only in regard to lowering the rate of multiples but also concerning the health of singletons (53).

The few economic analyses that do exist consistently demonstrate the greater patient, healthcare and societal costs associated with twins and higher-order multiples when compared with singleton infants, and convincingly add to the argument that single embryo transfer should be standard practice (54). A recent review of the key studies on the costs and consequences of ART treatment, specifically examined economic drivers of utilisation and clinical practice. The level of affordability of ART treatment is an important driver of treatment choices, embryo transfer practices and ultimately multiple birth rates (55). The decrease in multiple deliveries results in an improvement of the perinatal health of the IVF children and the wellbeing of the families. A healthy singleton delivery should be the goal of all IVF cycles, and this is best achieved by SET.

Practice points:

- Multiple birth rates after ART depend on the number of embryos transferred
- Twin birth rates still vary widely from country to country, from as low as 5 % to as high as 30%.
- Multiple pregnancies are associated with considerable risks for the mother and offspring as well as excess obstetric and neonatal costs
- Twin pregnancy rates can be lowered considerably with increasing use of elective single embryo transfer and cryopreservation of extra embryos
- Selection of the best embryo for transfer could be improved by using new techniques, including blastocyst culture, possibly by time-lapse monitoring or genetic assessment of the embryo

Research points:

- Future large cohort studies must publish complete datasets including cumulative live birth rates per started IVF stimulation
- Long follow-up studies on the health of children conceived with specified new techniques are important
- All new techniques must be studied both for safety and efficiency prior to routine adoption in the ART laboratory, including cost analyses of healthcare resources.
- Especially PGT-A must be addressed by further research include: cost-effectiveness; the role and effect of cryopreservation, utility in specific subgroups
- Large, prospective, well-controlled studies evaluating the combination of multiple approaches (genomics, time-lapse imaging, metabolomics, etc.) for embryo selection are needed

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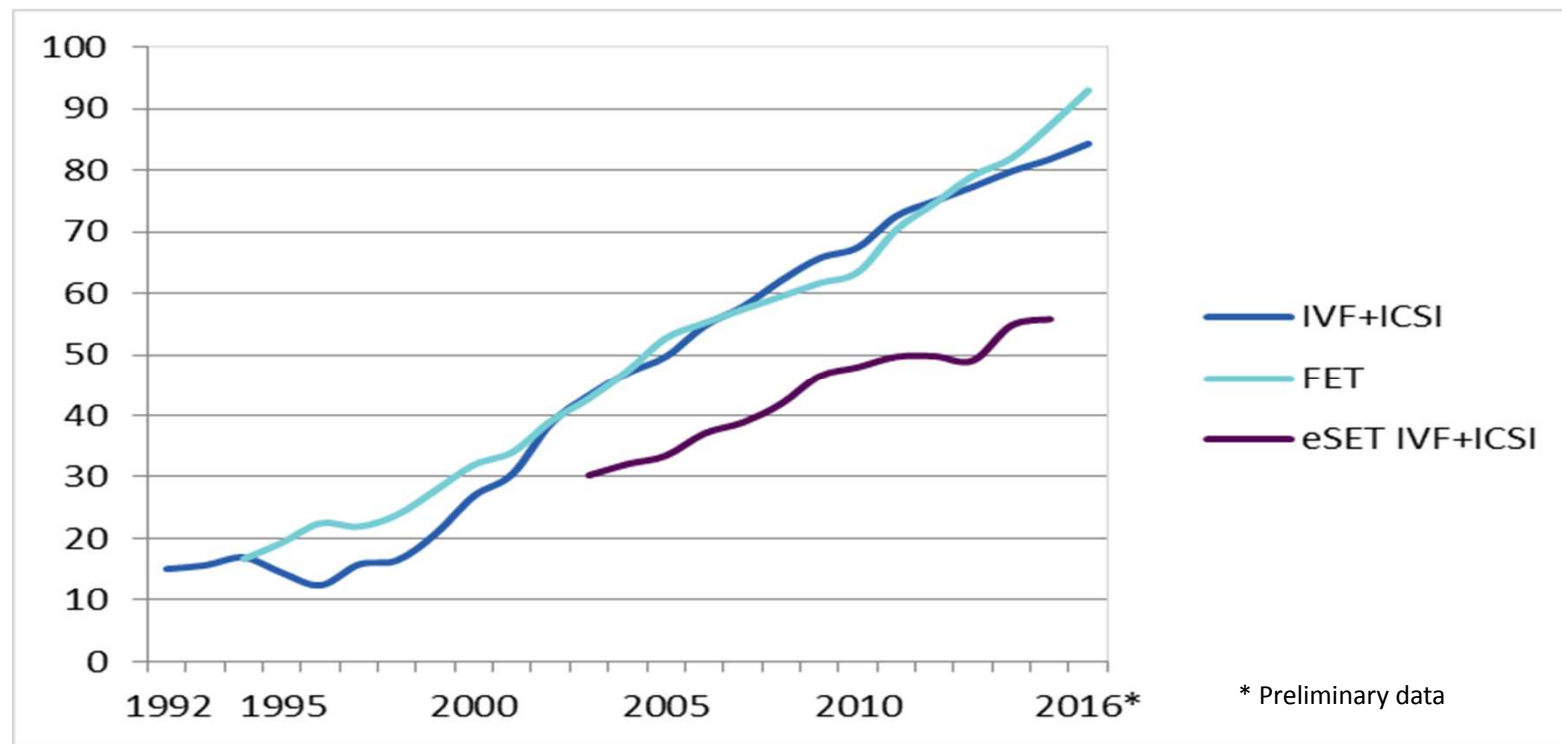
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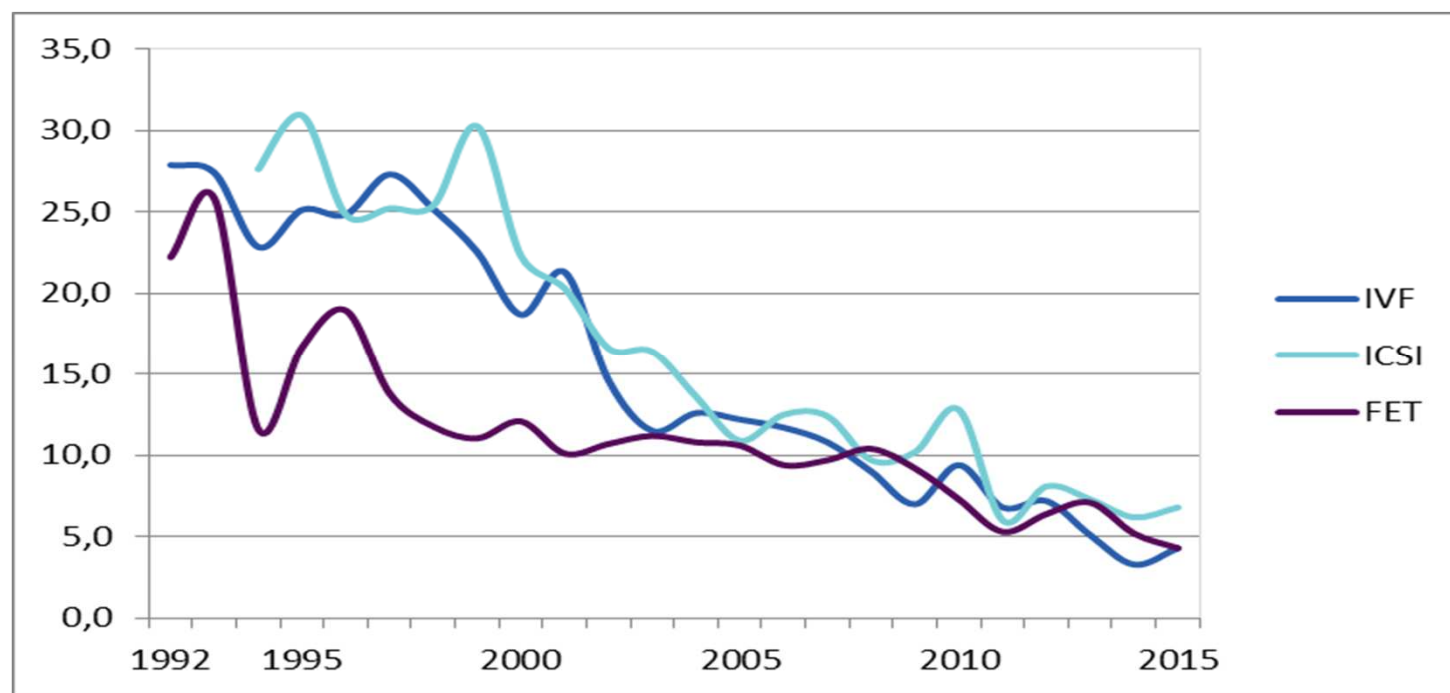
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Single embryo transfers, Finland 1992–2015, %



Source: THL, National Institute for Health and Welfare, Finland, Assisted Fertility Treatments

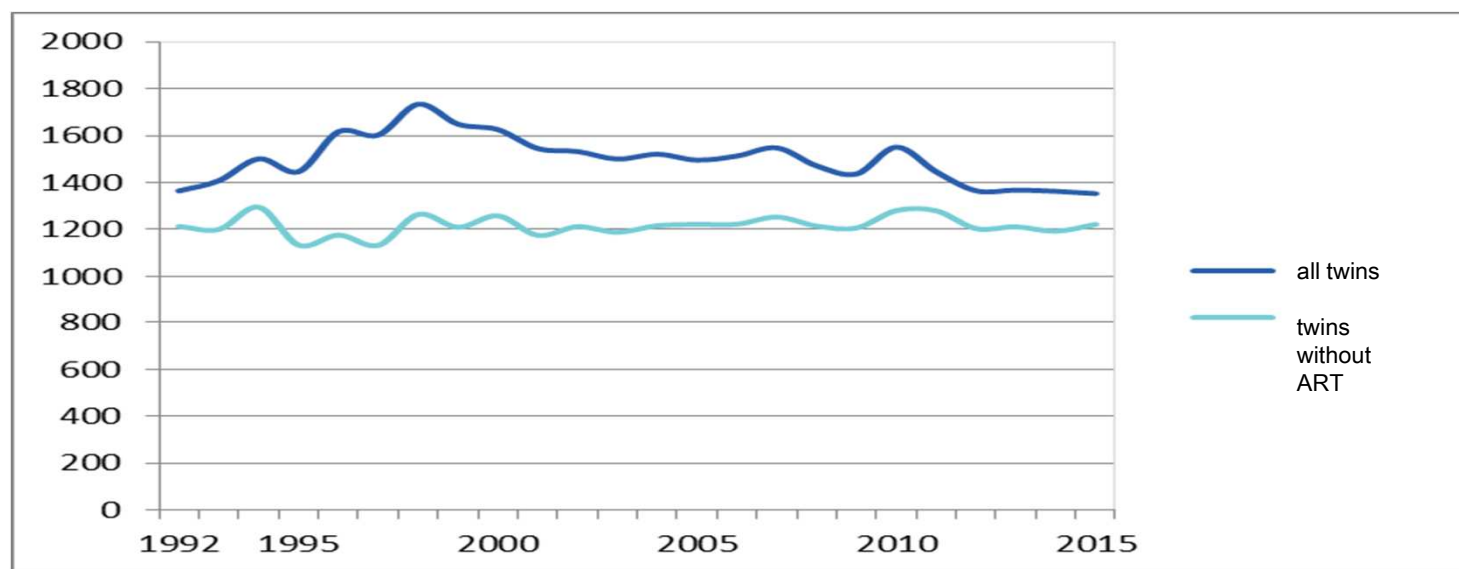
Proportion of multiple deliveries after ART, Finland, 1992–2015, %



Source: THL, National Institute for Health and Welfare, Finland, Assisted Fertility Treatments

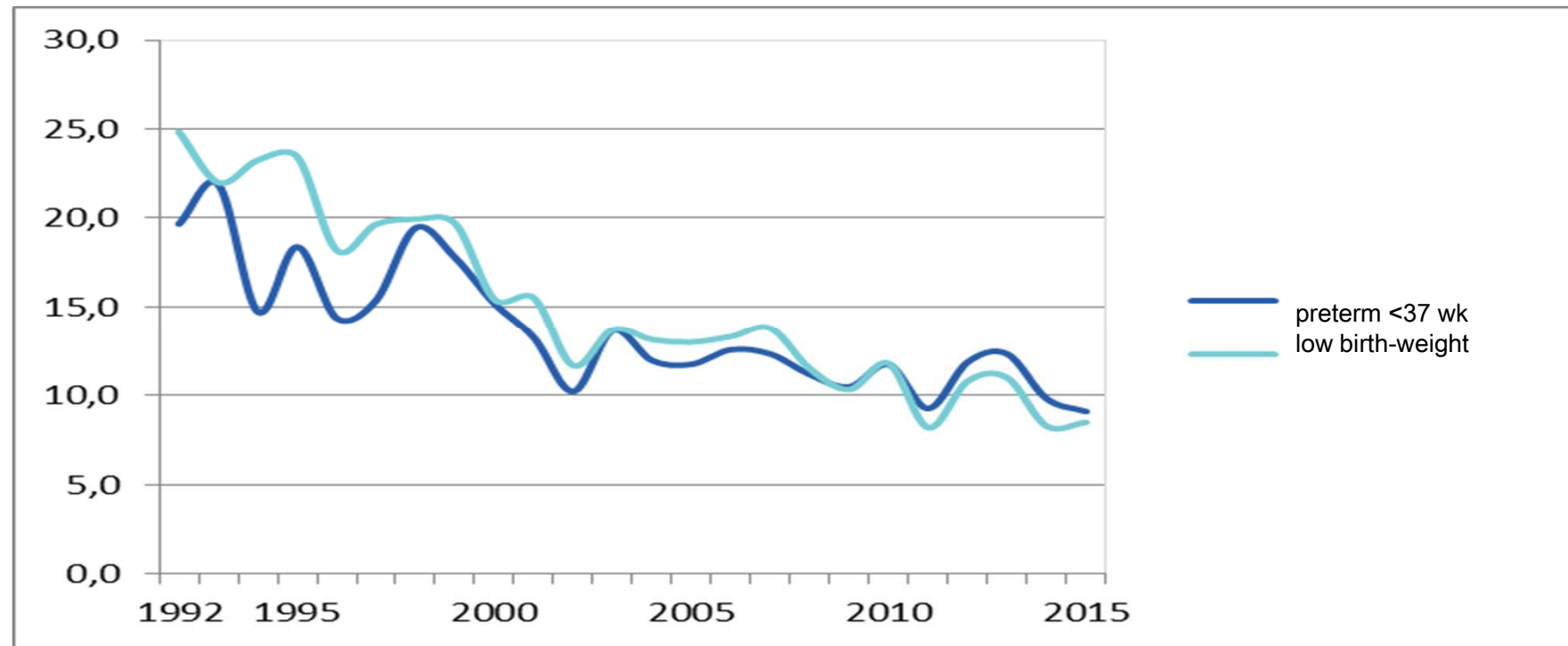
The effect of ART on the number of multiple deliveries, Finland, 1992–2015

Number of multiple deliveries / 100 000 deliveries



Source: THL, National Institute for Health and Welfare, Finland, Assisted Fertility Treatments, Medical Birth Registry

Proportion of preterm (<37 weeks) and low weight children (< 2500 g) in ART, Finland, 1992–2015, %



Source: THL, National Institute for Health and Welfare, Finland, Assisted Fertility Treatments